**Supplementary Tables and Figures**

**Supplementary Table S1.** Avian sampling. Complete list of fecal samples from wild bird species collected in both southern and northern Ukraine from December 2016 – December 2017. Sampling in the autumn (August-October) and winter (November-February) seasons were focused on collecting migrating birds, while spring season was focused on spring migration (March-May) and sampling prior to and after nesting period (June-July).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bird Species** | | **Seasons** | | |  |
| **Common Name** | **Scientific Name** | **Winter** | **Spring** | **Autumn** |  |
| ***ANSERIFORMES*** | | | | | **Total** |
| White-fronted Goose | *Anser albifrons* | 417 | 257 | 359 | 1033 |
| Red-breasted Goose | *Rufibrenta ruficollis* | - | - | 23 | 23 |
| Garganey | *Anas querquedula* | 25 | 18 |  | 43 |
| Mallard | *Anas platyrhynchos* | 253 | 415 | 432 | 1100 |
| Shelduck | *Tadorna tadorna* | 208 | 82 | 139 | 429 |
| Ruddy Shelduck | *Tadorna ferruginea* | 35 | 25 | 43 | 103 |
| Mute Swan | *Cygnus olor* | 11 | 23 | 15 | 49 |
| Whooper Swan | *Cygnus cygnus* | 125 | - | 145 | 270 |
| Greylag Goose | *Anser anser* | 32 | 340 | 14 | 386 |
| Wigeon | *Anas penelope* | 5 | - | 10 | 15 |
| Shoveler | *Anas clypeata* | 22 | 2 | - | 24 |
| Teal | *Anas crecca* | 10 | - | 51 | 61 |
| Wild duck | *Anas spp.* | 38 | - | - | 38 |
| Pintail | *Anas acuta* | 6 | - | - | 6 |
| ***CHARADRIIFORMES*** | | | | |  |
| Yellow-legged Gull | *Larus cachinnans* | 23 | 48 | 62 | 133 |
| Black-headed Gull | *Larus ridibundus* | 8 | 236 | 18 | 262 |
| Slender-billed Gull | *Larus genei* | 20 | 16 | - | 36 |
| Ruff | *Phylomachus pugnax* | 101 | 1 | - | 102 |
| Mediterranean Gull | *Larus melanocephalus* | 20 | 169 | - | 189 |
| Common Gull | *Larus canus* | 10 | - | 8 | 18 |
| Great Black-headed Gull | *Larus ichtyaetus* | - | 5 | 35 | 40 |
| Dunlin | *Calidris alpina* | - | - | 10 | 10 |
| Snipe | *Gallinago gallinago* | - | - | 15 | 15 |
| Little Gull | *Larus minutus* | - | 5 | - | 5 |
| Little Tern | *Sterna albifrons* | - | 5 | - | 5 |
| White-winged Black Tern | *Chlidonias leucopterus* | - | 20 | - | 20 |
| Caspian Tern | *Hydroprogne caspia* | 7 | - | - | 7 |
| Avocet | *Recurvirostra avosetta* | 5 | - | - | 5 |
| Gulls spp. |  | 4 | 10 | - | 14 |
| ***PODICIPEDIFORMES*** | | | | |  |
| Great Crested Grebe | *Podiceps cristatus* | - | - | 1 | 1 |
| ***GRUIFORMES*** | | | | |  |
| Demoiselle Crane | *Anthropoides virgo* | - | 12 | - | 12 |
| Crane | *Grus grus* | 16 | 17 | - | 33 |
| Coot | *Fulica atra* | 9 | - | - | 9 |
| ***CICONIIFORMES*** | | | | |  |
| Great Whitе Egret | *Egretta alba* | - | 15 | - | 15 |
| Little Egret | *Egretta garzetta* | - | 5 | - | 5 |
| Grey Heron | *Ardea cinerea* | - | 29 | - | 29 |
| Spoonbill | *Platalea leucorodia* | - | 7 | - | 7 |
| ***PELECANIFORMES*** | | | | |  |
| Dalmatian Pelican | *Pelecanus crispus* | - | 13 | 2 | 15 |
| Cormorant | *Phalacrocorax carbo* | - | 64 | 67 | 131 |
| White Pelican | *Pelecanus onocrotalus* | 15 | 26 | - | 41 |
| Pygmy Cormorant | *Phalacrocorax pygmaeus* | 5 | - | - | 5 |
| **FALCONIFORMES** | | | | |  |
| Marsh Harrier | *Circus aeruginosus* | - | 1 | - | 1 |
| ***OTHER*** | | | | |  |
| Environmental  (Fresh bird feces) | *Species was not identified* | - | 45 | - | 45 |
| **Total** | | **1430** | **1911** | **1449** | **4790** |

**Supplementary Table S2.** Overview of the infection rate of APMV-1 in wild birds by RT-PCR.

|  |  |
| --- | --- |
| **Species** | **Infection rate (%)** |
| Whooper Swan | 0.37 |
| Mallard | 0.36 |
| Mediterranean Gull | 0.52 |
| Shelduck | 0.46 |
| White-fronted Goose | 0.09 |
| White pelican  Snipe | 2.43  0.07 |

**Supplementary Table S3.** The isolation rate of APMV (avulavirus) in wild birds by virus isolation *in ovo*.

|  |  |
| --- | --- |
| **Species** | **Isolation rate (%)**  **APMV** |
| Greylag Goose | 0.26 |
| Mallard | 0.54 |
| Shelduck | 0.46 |
| Ruddy shelduck | 1.94 |

**Supplementary Table S4.** Overview of samples tested by HI assay from wild birds in 2017. AIV, avian influenza virus with provisional HA serological subtype noted, APMV avian paramyxovirus (avulavirus) with provisional type noted; Unidentified, samples that were HI positive (hemagglutinating) but not identifiable by serological panel available.

|  |  |  |
| --- | --- | --- |
| **No.** | **Strain name** | **Serological identification** |
| 1 | Shelduck/Baz Odesa/13-16-28-10/17 | AIV Н2/Н5/Н7 |
| 2 | Mallard/Er Odesa/41-50-8-08/17 | AIV Н3 |
| 3 | Mallard/Dr3 Kherson /1-5/6-08/17 | AIV Н3 |
| 4 | Ruddy shelduck/Pop Kherson/1-5-23-01/17 | AIV Н3 |
| 5 | Ruddy shelduck/Pop Kherson/6-10-23-01/17 | AIV Н3 |
| 6 | Ruddy shelduck/Pop Kherson/11-15-23-01/17 | AIV Н3 |
| 7 | Shelduck/Zh. Yar Odesa/1-5-27-10/17 | AIV Н3/Н7 |
| 8 | Environmental/ND Kherson/31-35-7-08/17 | AIV Н4 |
| 9 | Mallard/Ol2 Kherson/33-36/5-09/17 | AIV Н4 |
| 10 | Mallard/Dr3 Kherson /6-10-6-08/17 | AIV Н4 |
| 11 | Mallard/Zh. Yar Odesa/1-5/10-08/17 | AIV Н4 |
| 12 | Mallard/Syv Kherson/1-4/4-09/17 | AIV Н5 |
| 13 | Mallard/Chon Kherson/13-16/3-09/17 | AIV Н5 |
| 14 | Mallard/Hor Kherson/16-20/29-11/17 | AIV Н7/Н9 |
| 15 | Shelduck/Pop Kherson/21-25/23-01/17 | AIV Н10 |
| 16 | Whooper Swan/Des Odesa/1-5/30-01/17 | AIV Н11 |
| 17 | Whooper Swan/Des Odesa /6-10/30-01/17 | AIV Н11 |
| 18 | Black-headed Gull / Dr3 Kherson /1-3/6-08/17 | AIV Н11/Н13 |
| 19 | Greylag Goose /Myt Kherson/1-4/4-09/17 | APMV-1 |
| 20 | Mallard/Myt Kherson/1-4/4-09/17 | APMV-1 |
| 21 | Shelduck/Ser Kherson/11-15/6-08/17 | APMV-1 |
| 22 | Mallard/Kat Odesa/1-5/1-12/17 | APMV-1 |
| 23 | Mallard/Kat Odesa/6-10/1-12/17 | APMV-1 |
| 24 | Shelduck/Chur Kherson/1-5/2-11/17 | APMV-1 |
| 25 | Ruddy shelduck/Hr Kherson/11-15/27-01/17 | APMV-1/APMV-7 |
| 26 | Mallard/Hor Kherson/56-60/29-11/17 | APMV-4 |
| 27 | Mallard/Druzh Kherson/1-3/5-09/17 | APMV-4 |
| 28 | Environmental/ND Kherson /41-45/7-08/17 | APMV-6 |
| 29 | Mallard/ND Kherson /11-15/4-09/17 | APMV-6 |
| 30 | Mallard/Chon Kherson/41-45/1-11/17 | AIV Н1 |
| 31 | Ruddy shelduck/ ND Kherson /1-5/5-08/17 | Unidentified |
| 32 | Mallard/Prym T Odesa/35-39/26-09/17 | Unidentified |
| 33 | White fronted goose/Vas Kherson/2,4,5/29-11/17 | Unidentified |
| 34 | Environmental/ND Kherson /26-30/7-08/17 | Unidentified |
| 35 | Shelduck/Ser Kherson/16-20/6-08/17 | Unidentified |
| 36 | Mallard/Dr2 Kherson /16-20/6-08/17 | Unidentified |
| 37 | Mallard/Ras Odesa/36-40/11-08/17 | Unidentified |
| 38 | Mallard/Ras Odesa/16-20/11-08/17 | Unidentified |
| 39 | Mallard/PK Kherson/1-5/7-08/17 | Unidentified |
| 40 | Mallard/Vas Kherson/1-5/24-01/17 | Unidentified |

**Supplementary Table S5.** APMV Tiling primer sequences and pooling used to generate APMV-1 and APMV-6 amplicons for nanopore sequencing. Primer .xlsx table attached.

**Supplementary Table S6.** APMV Reference sequences representative from each APMV serotype in reference database used for virus genome assembly.

**Supplementary Table S7.** Overview of 2017 APMV-1 and APMV-6 positive samples sequenced using MinION. APMV was amplified using a RT PCR with four pools of tiling primers. Amplicons were sequenced with an LSK109 nanopore sequencing kit. Sequences were aligned to a reference database to identify a closest match for further analysis with Minimap2. Consensus sequences were generated using Medaka. Sequences were realigned to the reference and the percent identity was calculated with Geneious.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample Identifier** | **Species** | **Sample Location** | **Total # of reads sequenced** | **Closest Reference** | **Percent Identity** | **Consensus Sequence Length** |
| APMV1|Mallard|Myt\_Kherson|1-4-4-09|2017 | Mallard | Myt Kherson | 92,095 | KX352836|APMV1|Teal|Russia|2010\_08\_28 | 98.4% | 14,089 |
| APMV1|Mallard|AN\_Kherson|TM434778|2002 | Mallard | Kherson | 653,240 | KX352836|APMV1|Teal|Russia|2010\_08\_28 | 98.7% | 15,168 |
| APMV-6|Environmental|ND Kherson|41-45|7-08|2017 | Env. | Kherson | 429,606 | JX522537.1|APMV6|  Mallard|China|2011 | 98.6% | 16,234 |
| APMV-6|Mallard|ND Kherson|11-15|4-09|2017 | Mallard | Kherson | 764,971 | JN571486|APM6|  Duck|Belgium|2007 | 96.6% | 16,235 |
| APMV-1|Mallard|Dr\_Kherson|1-3|5-09|2017 | Mallard | Druzh Kherson | 121,493 | KU601398|APMV1|Auk|Russia|2015 | 98.7% | 15,085 |
| APMV-1|Grey\_Goose|Myt|1-4|4-09|2017 | Grey goose | Myt Kherson | 925,542 | KU601398|APMV1|Auk|Russia|2015 | 99.1% | 15,093 |
| APMV-1|Shelduck|Chur\_Kherson|1-5|2-11|2017 | Shelduck | Chur Kherson | 1,276,200 | KU601398|APMV1|Auk|Russia|2015 | 99.2% | 15,090 |

**Supplementary Table S8.** Amino acid substitution table for

APMV1/Mallard/Myt Kherson/1-4/4-09/17 compared to reference KX352836|APMV1|Teal|Russia|2010\_08\_28.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleoprotein** | | **Phosphoprotein** | | **Matrix protein** | | **Fusion Protein** | | **Hemagglutinin Protein** | | **Large Polymerase Protein** |
| Y230H | | T210I | | K226R | | None | | L211P | | S3G |
|  |  | S239N | | G230R | |  |  | RR 293G | | V187I |
|  |  | I248V | |  |  |  |  | E495V | | V207E |
|  |  | A341V | |  |  |  |  | C596Y | | I661V |
|  |  | M342I | |  |  |  |  | K603E | | S894F |
|  |  |  |  |  |  |  |  |  |  | S952N |
|  |  |  |  |  |  |  |  |  |  | R1071K |
|  |  |  |  |  |  |  |  |  |  | N1564H |
|  |  |  |  |  |  |  |  |  |  | S1753N |
|  |  |  |  |  |  |  |  |  |  | weird |
|  |  |  |  |  |  |  |  |  |  | weird |
|  |  |  |  |  |  |  |  |  |  | weird |
|  |  |  |  |  |  |  |  |  |  | T2095I |
|  |  |  |  |  |  |  |  |  |  | L2139P |
|  |  |  |  |  |  |  |  |  |  | weird |
|  |  |  |  |  |  |  |  |  |  | weird |
|  |  |  |  |  |  |  |  |  |  | D2053N |
|  |  |  |  |  |  |  |  |  |  | weird |

**Supplementary Table S9.** Amino acid substitution table for

APMV-1/Mallard/AN Kherson/TM434778/2002 compared to reference KX352836|APMV1|Teal|Russia|2010\_08\_28.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleoprotein** | | **Phosphoprotein** | | | **Matrix Protein** | | **Fusion Protein** | | **Hemagglutinin Protein** | | | **Large Polymerase Protein** |
| None | | A78V | | None | | | T203A | | | R63K | | S3G |
|  |  |  |  |  | |  |  |  | | C123S | | S156C |
|  |  |  |  |  | |  |  |  | | D285E | | V187I |
|  |  |  |  |  | |  |  |  | | R293G | | O626P |
|  |  |  |  |  | |  |  |  | | K567R | | S894F |
|  |  |  |  |  | |  |  |  | | I590V | | E1031G |
|  |  |  |  |  | |  |  |  | | K603E | | P1734S |
|  |  |  |  |  | |  |  |  | |  |  | Y2202C |

**Supplementary Table S10.** Amino acid substitution table for

APMV-6/Environmental/ND Kherson/41-45/7-08/17 compared to reference JX522537.1|APMV6|Mallard|China|2011.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleoprotein** | | **Hemagglutinin Protein** | | **Phosphoprotein** | | | **Matrix protein** | | **Fusion Protein** | | **Large Polymerase Protein** | | **SH Protein** | |
| A79T | | K57R | | S64P | | V342I | | | N20S | | T51A | T49A | | | |
| D419N | | P64S | | S68P | |  | | | A76T | | I121V | N89T | | | |
|  |  | S71G | | N83D | |  | | | S77G | | A405T |  | |  | |
|  |  | V171A | | H168Y | |  | |  | E148D | | S637N |  | |  | |
|  |  | V212I | |  |  |  | |  |  |  | P650L/M |  | |  | |
|  |  | R356G | |  |  |  | |  |  |  | H725N |  | |  | |
|  |  | C438T | |  |  |  | |  |  |  | P939L |  | |  | |
|  |  | I446L/M | |  |  |  | |  |  |  | Q1097H |  | |  | |
|  |  | A481V | |  |  |  | |  |  |  | N1269K |  | |  | |
|  |  | F560S | |  |  |  | |  |  |  | Y1599C |  | |  | |
|  |  | T604A | |  |  |  | |  |  |  | V2031A |  | |  | |
|  |  |  |  |  |  |  | |  |  |  | Q2099H |  | |  | |
|  |  |  |  |  |  |  | |  |  |  | L2229F |  | |  | |

**Supplementary Table S11.** Amino acid substitution table for

APMV-6/Mallard/ND Kherson/11-15/4-09/17 compared to reference JN571486|APM6|Duck|Belgium|2007.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleoprotein** | | **Hemagglutinin Protein** | **Phosphoprotein** | **Matrix protein** | **Fusion Protein** | **Large Polymerase Protein** | **SH protein** |
| G50S | V34I | | T60I | I48V | L8P | R131Q | V20G |
|  | I63V | | S78I |  | R26G | N176S | I38V |
|  | E106K | | G82E |  | E98D | R207K | G39R |
|  | N153S | | S84L |  |  | E294G | N90D |
|  | V205A | | P94L |  |  | E322D |  |
|  | V226M | | P108L/M |  |  | I456V |  |
|  | D293N | | T117X |  |  | K502R |  |
|  | Q315H | | N118H |  |  | L631F |  |
|  | L339F | | T150A |  |  | T643K |  |
|  | K414R | | A166T |  |  | P644S |  |
|  | G596M | | T175A |  |  | T648A |  |
|  | Y603H | | D196E |  |  | D726N |  |
|  |  | | E206D |  |  | P937L |  |
|  |  | | F246L |  |  | L1059Q |  |
|  |  | | A348T |  |  | S1400I |  |
|  |  | | I379T |  |  | N1814H |  |
|  |  | | T380M |  |  | P2080S |  |
|  |  | | N415S |  |  |  |  |

**Supplementary Table S12.** Amino acid substitution table for

APMV-1/mallard/Dr Kherson/1-3/5-09/17 2017 compared to reference

KU601398|APMV1|Auk|Russia|2015.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleoprotein** | | **Hemagglutinin Protein** | | **Phosphoprotein** | | **Matrix protein** | | **Fusion Protein** | | **Large Polymerase Protein** |
| Y489H | | L211P | | V78A | | S48L | | I335T | | K263R |
|  |  | I270M | | S239N | | K256R | |  |  | I417V |
|  |  | E495V | | I248V | | G230R | |  |  | O626P |
|  |  | C596Y | | A341V | |  | |  |  | I661V |
|  |  | A598T | | M342I | |  |  |  |  | S952N |
|  |  |  |  |  |  |  |  |  |  | V1365M |
|  |  |  |  |  |  |  |  |  |  | N1564H |

**Supplementary Table S13.** Amino acid substitution table for

APMV-1/Grey goose/Myt/1-4/4-09/17 compared to reference

KU601398|APMV1|Auk|Russia|2015.

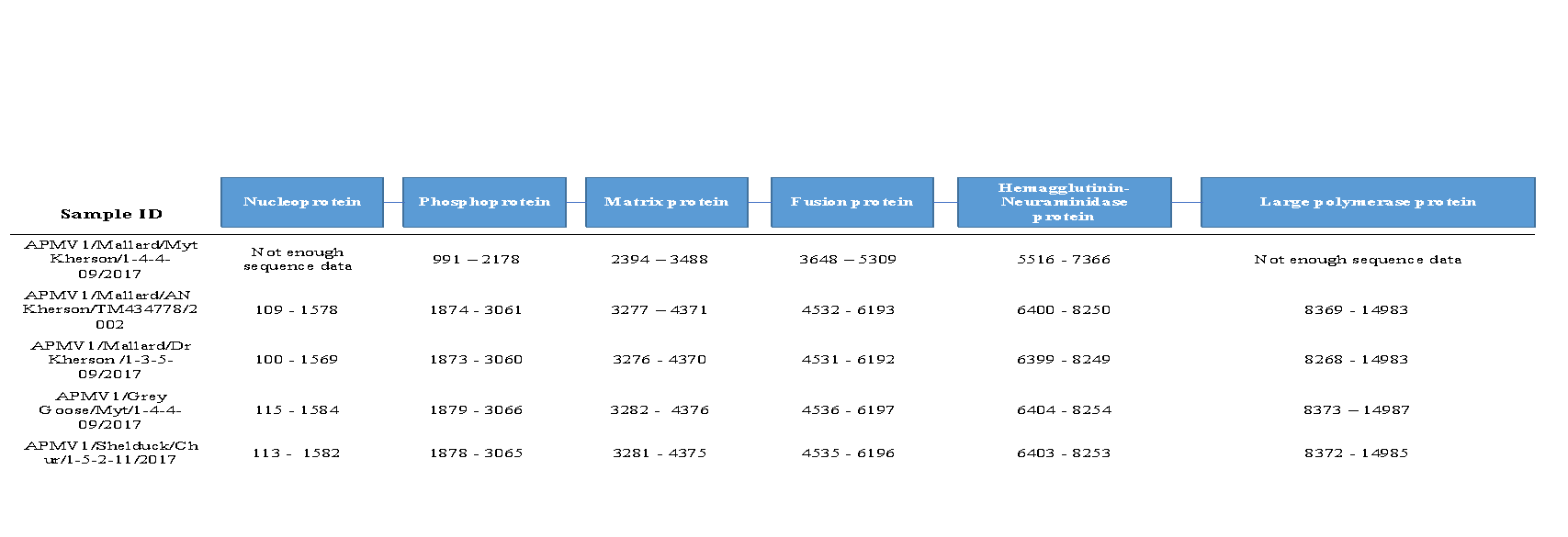
|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleoprotein** | | **Hemagglutinin Protein** | | **Phosphoprotein** | | **Matrix protein** | | **Fusion Protein** | | **Large Polymerase Protein** |
| Y489H | | I270M | | V78A | | K226R | | I335T | | K263R |
|  |  | E495K | | T210I | | G230R | |  |  | I417V |
|  |  | C596Y | | S239N | |  | |  |  | O626P |
|  |  | A598T | | A341V | |  | |  |  | I661V |
|  |  |  | | M342I | |  |  |  |  | S952N |
|  |  |  |  |  |  |  |  |  |  | V1365M |
|  |  |  |  |  |  |  |  |  |  |  |

**Supplementary Table S14.** Amino acid substitution table for

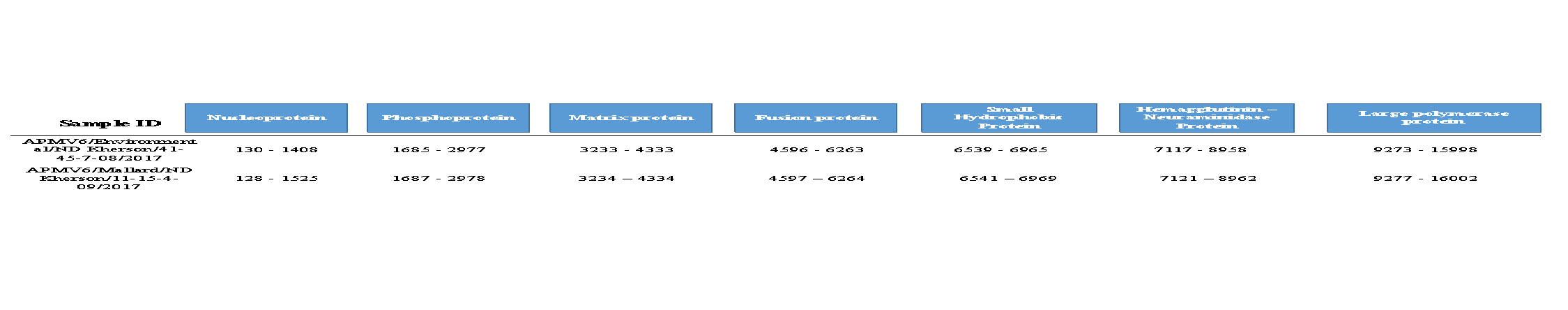
APMV-1/shelduck/Chur/1-5/2-11/17 compared to reference

KU601398|APMV1|Auk|Russia|2015

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleoprotein** | | **Hemagglutinin Protein** | | | **Phosphoprotein** | | **Matrix protein** | | **Fusion Protein** | | **Large Polymerase Protein** |
| N463S | | I270M | | none | | | T74I | | V19A | | T253M |
|  |  | A598T | |  | |  |  |  | I335T | | I417V |
|  |  |  |  |  | |  |  |  |  |  | K1071R |
|  |  |  |  |  | |  |  |  |  |  | V1365M |
|  |  |  |  |  | |  |  |  |  |  | M2056T |

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**Supplementary Figure S1.** Genome assembly of APMV-1 sequences isolated in this study. Reads were aligned to APMV-1 specific reference database to identify a closest reference using Minimap2 and a consensus sequence was generated using Medaka. Consensus sequence was re-aligned to the reference to generate genome annotations using Geneious v.11.0.3.

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**Supplementary Figure S2.** Genome assembly of APMV-6 sequences isolated from this study. Reads were aligned to APMV-6 specific reference database to identify a closest reference using Minimap2 and a consensus sequence was generated using Medaka. Consensus sequence was re-aligned to the reference to generate genome annotations using Geneious v11.0.3.